BRIEF COMMUNICATIONS AAEM

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PREVALENCE OF ANTIBODIES TO *ENCEPHALITOZOON CUNICULI* (MICROSPORIDIA) IN ANGORA GOATS – A POTENTIAL RISK OF INFECTION FOR BREEDERS

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Abstract: The presence of antibodies against *Encephalitozoon cuniculi* in Angora goats was detected by the method of indirect immunofluorescence (IFAT). The animals reacting at the titre 1: 64 and more were considered positive. Of the total number of 48 sera examined, 4 were positive at the titre 1: 32 and 2 were positive at the titre 1: 64. The occurrence of antibodies against *E. cuniculi* indicates that one of the causes of disorders in the reproductive cycle in Angora goats may be microsporidia *Encephalitozoon cuniculi*, and that these animals may be potential sources of infection for people.

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INTRODUCTION

Encephalitozoonosis is caused by an intracellular eukaryote protozoan parasite *Encephalitozoon cuniculi* [9], belonging to the phylum Microspora of the family Encephalitozoonidae [18]. The development of the parasite occurs only in living host cells with active metabolism (endothelial cells and macrophages) inside the parasitophorous vacuoles.

Encephalitozoon cuniculi infects a wide range of mammalian hosts, including rodents [24], rabbits [10], dogs [20, 21], blue foxes [14], cows [6], and nonhuman primates [25]. In goats, *Encephalitozoon cuniculi (Nosema cuniculi)* was first described by Khanna and Iyer [8]. *Encephalitozoon cuniculi* has been also described as an opportunistic pathogen in patients with decreased resistance, e.g. in patients with AIDS [3, 23]. Recently, Mathis *et al.* [11] has found a rabbit *Encephalitozoon cuniculi* isolate, classified by Didier *et al.* [4] as strain I, in human urine, confirming the zoonotic character of the disease.

A characteristic feature of the infection induced by *Encephalitozoon cuniculi* in susceptible hosts is usually its chronic latent course, with significantly higher mortality in young and older individuals. Latently infected hosts, given immunosuppression factors, will develop an acute, clinically apparent disease [16].

The most common modes for transmitting infections with *Encephalitozoon* spp. are ingestion and inhalation. Transplacental transmission of infection may also play an important role in the epizootiology of encephalitozoonosis, especially in carnivores and rodents [13].

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The Angora goats investigated for the presence of antibodies against *E. cuniculi* were from intensive goat breeding. The abortions, stillbirths and births of weak kids were observed in this breeding.

The reproductive disorders and abortions in goats may be caused by a number of factors – a metabolic derangement, e.g. iodine deficiency [1], wrong zoohygienic conditions, and also bacterial, viral and parasitic infectious agents. One of the infectious agents responsible for infertility and abortions of goats may also be microsporidia *Encephalitozoon cuniculi* from the kingdom Protista. It is also an important fact that goats infected by microsporidia *Encephalitozoon cuniculi* may pose a potential source of infection for people, especially for breeders attending these animals.

MATERIALS AND METHODS

Goats. Forty-eight dams of Angora goats, imported originally from Denmark, were examined. The goats were grazed in the summer periods of 1994 and 1995 and housed permanently in a barn with access to a walk in 1996 and 1997. The major health problem in 1994 and 1996 was a high incidence of reproductive failures, including abortions, stillbirths and births of weak kids.

Sera. Blood samples were withdrawn from the *vena jugularis.* The serum samples prepared were stored at -20ºC until serological examination.

Antigen of *Encephalitozoon cuniculi.* *Encephalitozoon cuniculi* parasites were grown in E6 cells (VERO green monkey kidney cells) for the provision of spores. The infected cells were cultivated in modified RPMI 1640 medium, supplemented with 5% foetal calf serum and the addition of antibiotics and antimycotics (penicillin, streptomycin and amphotericin B). After lysis, the infected cells broke and spores were released into the medium from which they were separated by centrifugation at 400 × *g* for 30 min. After rinsing in Percoll, they were again centrifuged and stored at 4ºC.

The indirect immunofluorescence antibody test (IFAT). The IFAT method was used to determine specific anti-*Encephalitozoon cuniculi* antibodies. The method was performed according to Chalupský *et al.* [2].

Fresh suspension of *Encephalitozoon cuniculi* from tissue culture was placed on each well of a slide. The slides were air-dried for 24 h, then fixed in absolute acetone for 15 min and air-dried. The goats' sera tested were serially diluted beginning at 1: 16 and ending at 1: 256. Each of the wells on the slide was covered with 10 µl of rabbit anti-goat immunoglobulin plus fluorescein isotiocyanate conjugate (Sigma, Saint Louis, USA) of 1: 160 dilution. After 30 min at 37ºC the slides were washed and air-dried. They then were counter-stained with Evans blue and coverslips mounted with buffered glycerine.

The animals whose sera reacted at a dilution of 1: 64 or higher were considered to be positive.

RESULTS

Of 48 serum samples from the angora goats assayed by IFAT, 4 were positive at the titre 1:16 (8,3%) and 2 were positive at the titre 1:64, which represented 4.1%. In positive cases, spores of *Encephalitozoon cuniculi* were shown as oval, fluorescent formations of 1.5 µm × 2.5 µm. At the titre 1:128 and higher the animals were negative.

DISCUSSION

Encephalitozoon cuniculi, originally detected in rabbits [24] is an obligate intracellular protozoan parasite, invading various species of the animal kingdom, including the goat. It was the first mammal microsporidian cultivated *in vitro* [17].

E. cuniculi has been known as an agent causing encephalitozoonosis, a chronic disease, usually with asymptomatic progression [19] involving the central and peripheral nervous system, kidneys and ureters. In 1995, the zoonotic character of encephalitozoonosis was proved [3] and microsporidia became of interest not only for veterinary but also for human medicine.

In goats, *E. (Nosema) cuniculi* was found in the tubules of the kidney which showed changes associated with a focal chronic interstitial nephritis [8]. Similar findings were also observed in the kidneys of mice [5], rabbits [7], dogs [12] and cows [15] suffering from encephalitozoonosis. By IFAT, the *E. cuniculi* infection in goats was first described by Waller *et al.* [22] in Sweden.

In 1995, Reetz [15] published a report on the immunohistochemical evidence of the microsporidia antigens, probably *E. cuniculi,* in two cases of abortions in cattle in the seventh month of pregnancy. Microbiological examination of foetuses and foetal placentas did not reveal any of the usual bacterial or mycotic agents of abortion.

The results of our serological examination have confirmed the occurrence of antibodies against *E. cuniculi* in goats. In this breed there were observed abortions, stillbirths and births of weak kids. Although the percentage of positive reactions showing the presence of antibodies against antigens of *E. cuniculi* was not high (4,1%), it indicates that one of the possible causes of reproductive disorders may also be microsporidia *Encephalitozoon cuniculi.* Thus, breeders and veterinarians may be exposed during deliveries to the transmission of pathogens from infected animals. Breeders could also be infected by cleaning and tending the animals, because the transmission by contact by hand contaminated with urine containing spores of *E. cuniculi* was also described.

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